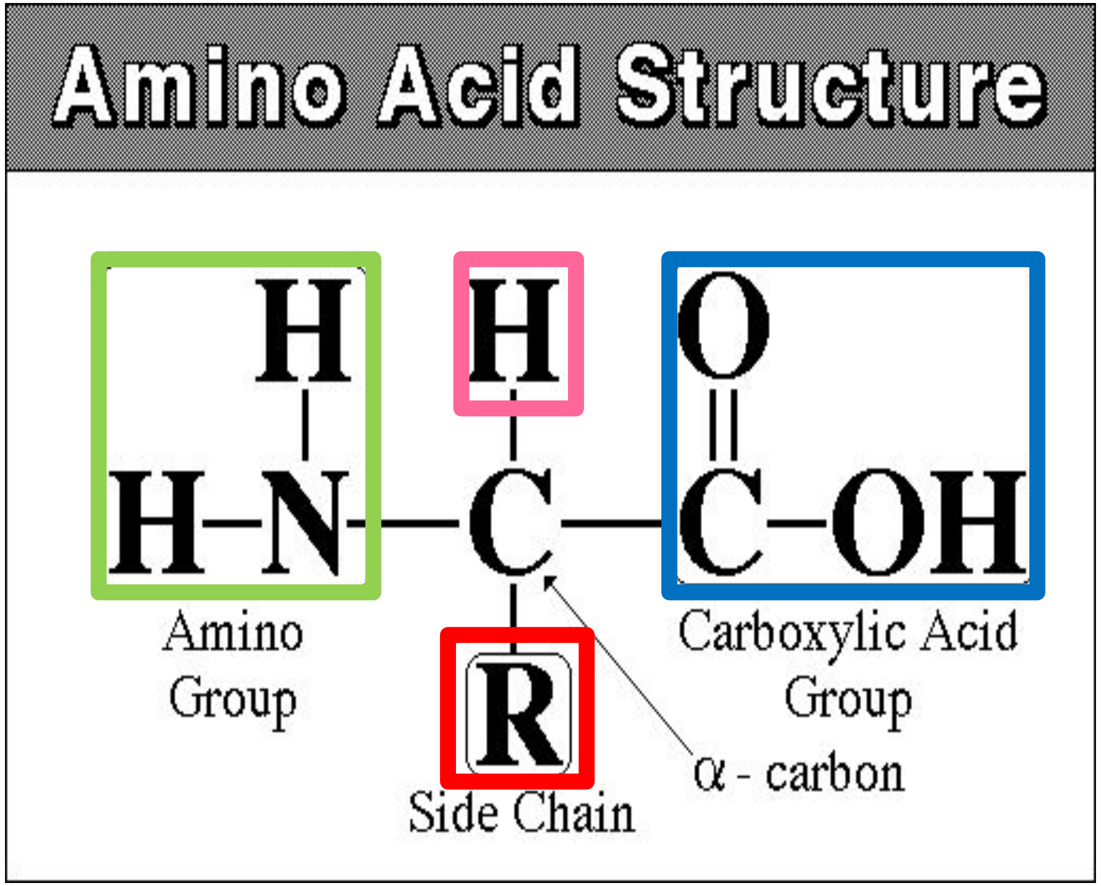
Titration curve of amino acids

Titration Curves :

- Titration Curves are produced by monitoring the pH of a given volume of a sample solution after successive **addition of acid or alkali**.
- The curves are usually plots of pH against the volume of titrant added (acid or base).
- Each dissociation group represent **one stage** in the titration curve.

Amino acid general formula:

- **Amino acids consist of:**
  - A basic amino group ($—NH_2$)
  - An acidic carboxyl group (—COOH)
  - A hydrogen atom (—H)
  - A distinctive side chain (—R).



Titration of amino acid:

- When an amino acid is dissolved in water it exists predominantly in the **isoelectric form.**

- Amino acid is an **amphoteric** compound➔ It act as either an acid or a base:

  - **Upon titration with acid** ➔ it acts as a BASE (accept a proton).
  - **Upon titration with base** ➔ it acts as an ACID (donate a proton)

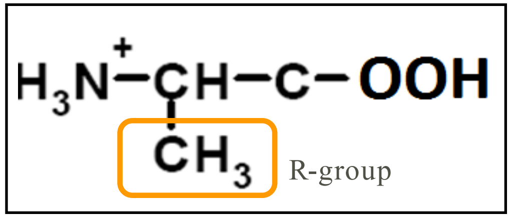
Titration of amino acid cont':

- Amino acids are example of **weak acid** which contain **more than one dissociate group.**
- **Examples:**

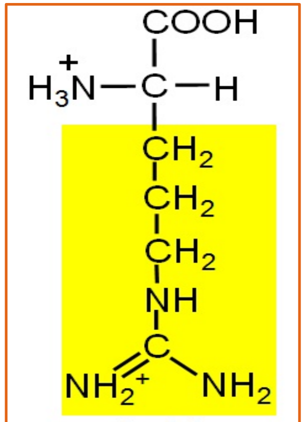
(1) **Alanine:**

-Contain COOH (pKa_1= 2.34) and NH_3^+ (pKa_2= 9.69) groups (it has one pI value =6.010). [Diprotic]

-The COOH will dissociate first then NH_3^+ dissociate later . (Because pKa_1<pKa_2)



Full protonated alanine

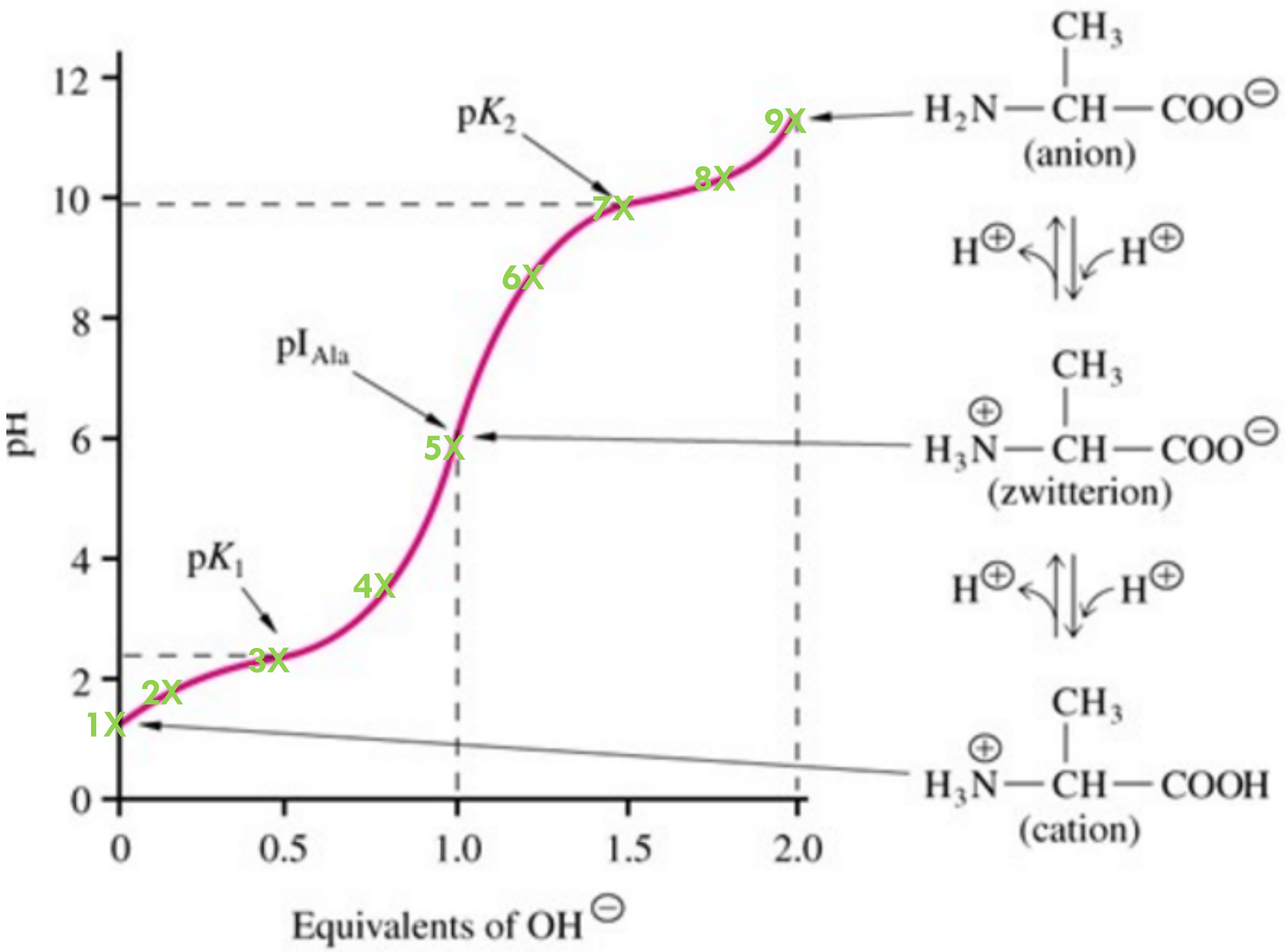


Arginine

(2) **Arginine:**

-Contain COOH (pKa_1= 2.34) , NH_3^+ (pKa_2= 9.69) groups and basic group (pKa_3=12.5) (it has one pI value=11). [Triprotic]

Titration curve of Alanine



pK_1 carboxylic acid = 2.34

pK_2 amino group = 9.69

$pI = (pK_1 + pK_2)/2$

Titration curve of alanine or glycine [diprotic]:

[1] In starting point:

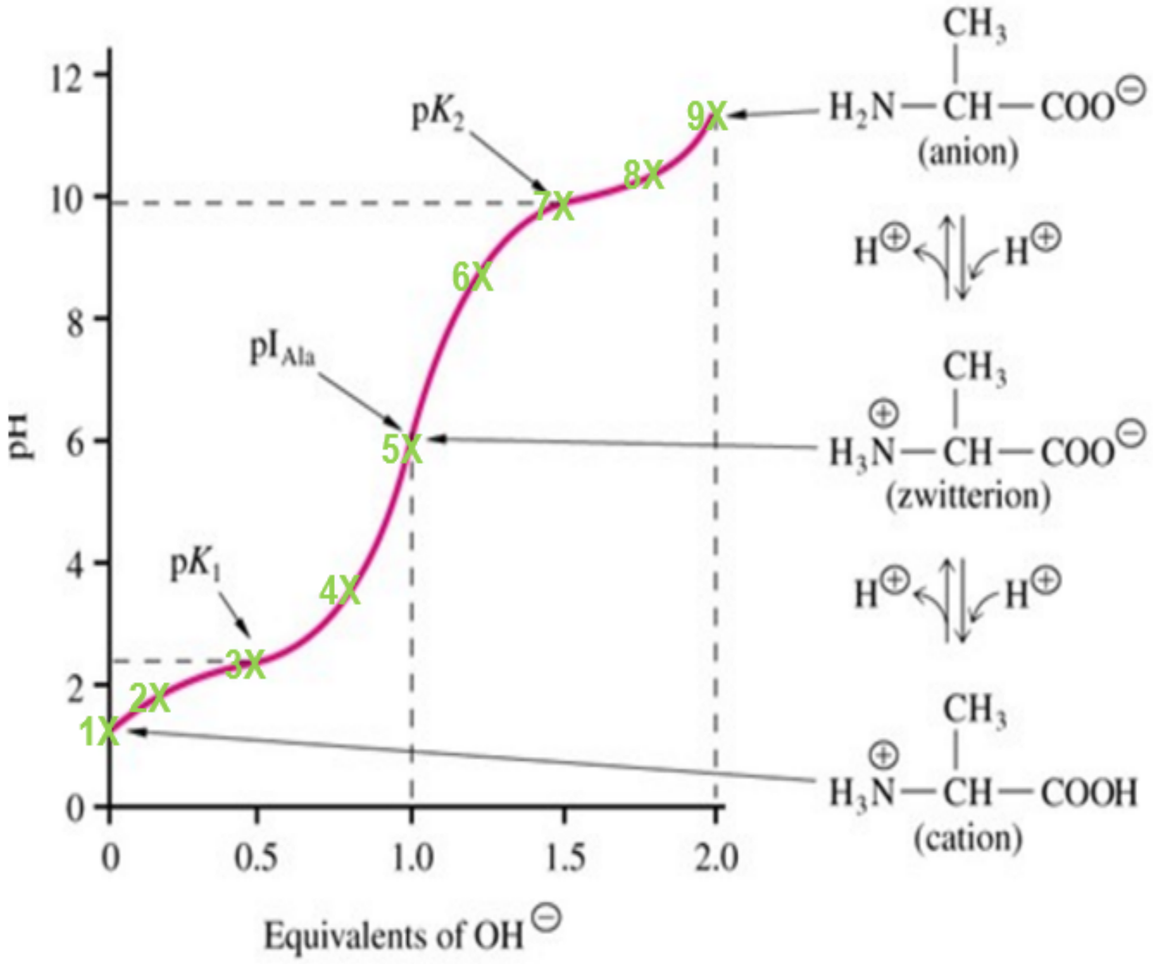
- Alanine is full protonated.
- [NH_3^+-CH-CH_3-COOH].

[2] COOH will <u>dissociate</u> first:

- [NH_3^+-CH-CH_3-COOH] > [NH3+-CH-CH3-COO^-]
- pH<pKa_1.

[3] In this point the component of alanine act as buffer:

- [NH_3^+-CH-CH_3-COOH]=[NH_3^+-CH-CH_3-COO^-].
- pH=pKa_1



Titration curve of alanine or glycine [diprotic]:

[4] In this point:

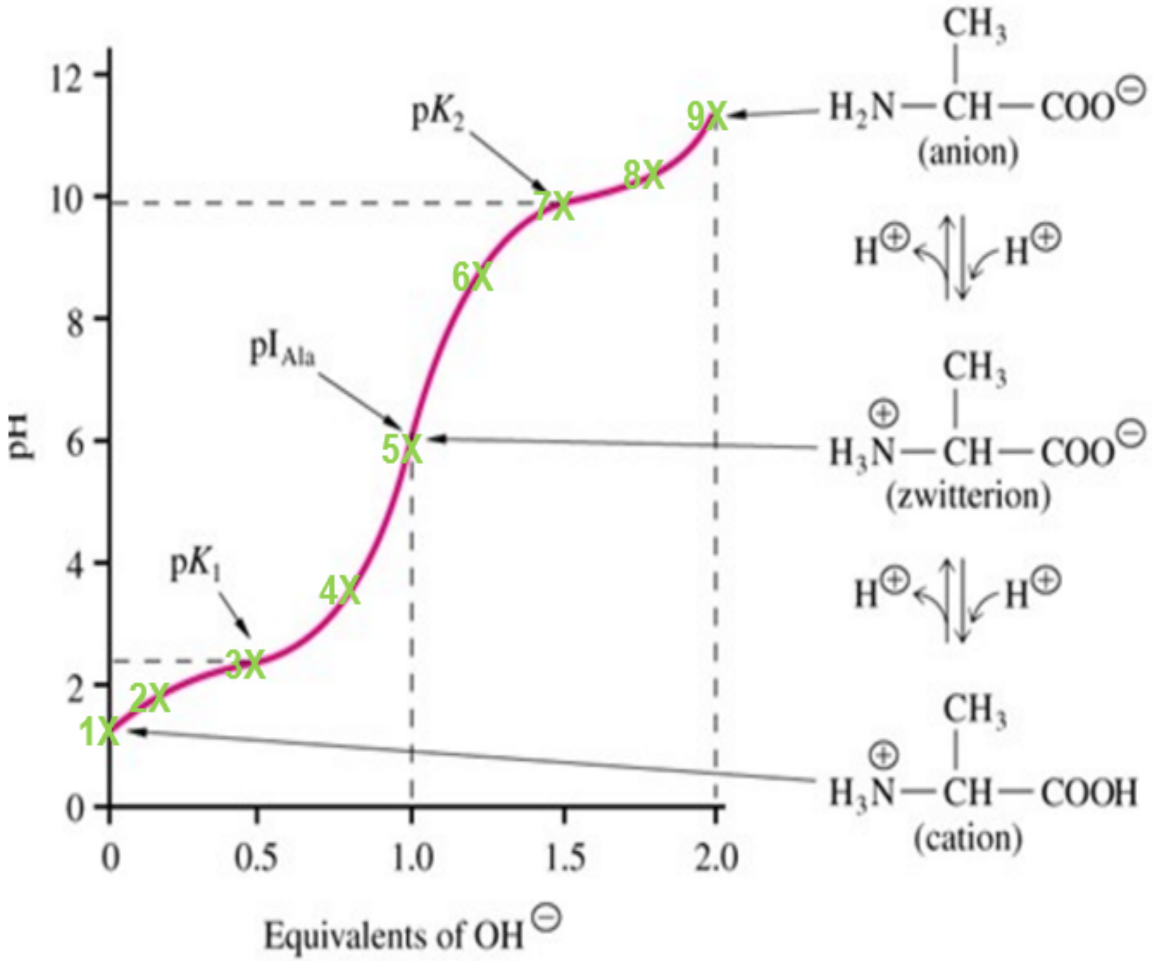
- $[NH_3^+\text{-CH-CH}_3\text{-COOH}] < [NH_3^+\text{-CH-CH}_3\text{-COO}^-]$.
- $pH > pKa_1$.

[5] Isoelectric point:

- The COOH is full dissociate to COO^-.
- $[NH_3^+\text{-CH-CH}_3\text{-COO}^-]$.
- Con. of -ve charge = Con. of +ve charge.
- The amino acid present as Zwetter ion (neutral form) .
- <u>Remember that</u> :PI (isoelectric point) is the pH value at which the net charge of amino acid equal to zero.
- $pI = (pKa_1 + pKa_2)/2 = (2.32+9.96)/2 = 6.01$

[6] The NH_3^+ start <u>dissociate</u>:

- $[NH_3^+\text{-CH-CH}_3\text{-COO}^-] > [NH_2\text{-CH-CH}_3\text{-COO}^-]$.
- $pH < pKa_2$.



Titration curve of alanine or glycine [diprotic]:

[7] In this point the component of alanine act as buffer:

- $[NH_3^+\text{-CH-CH}_3\text{-COO}^-] = [NH_2\text{-CH-CH}_3\text{-COO}^-]$.
- $pH = pKa_2$.

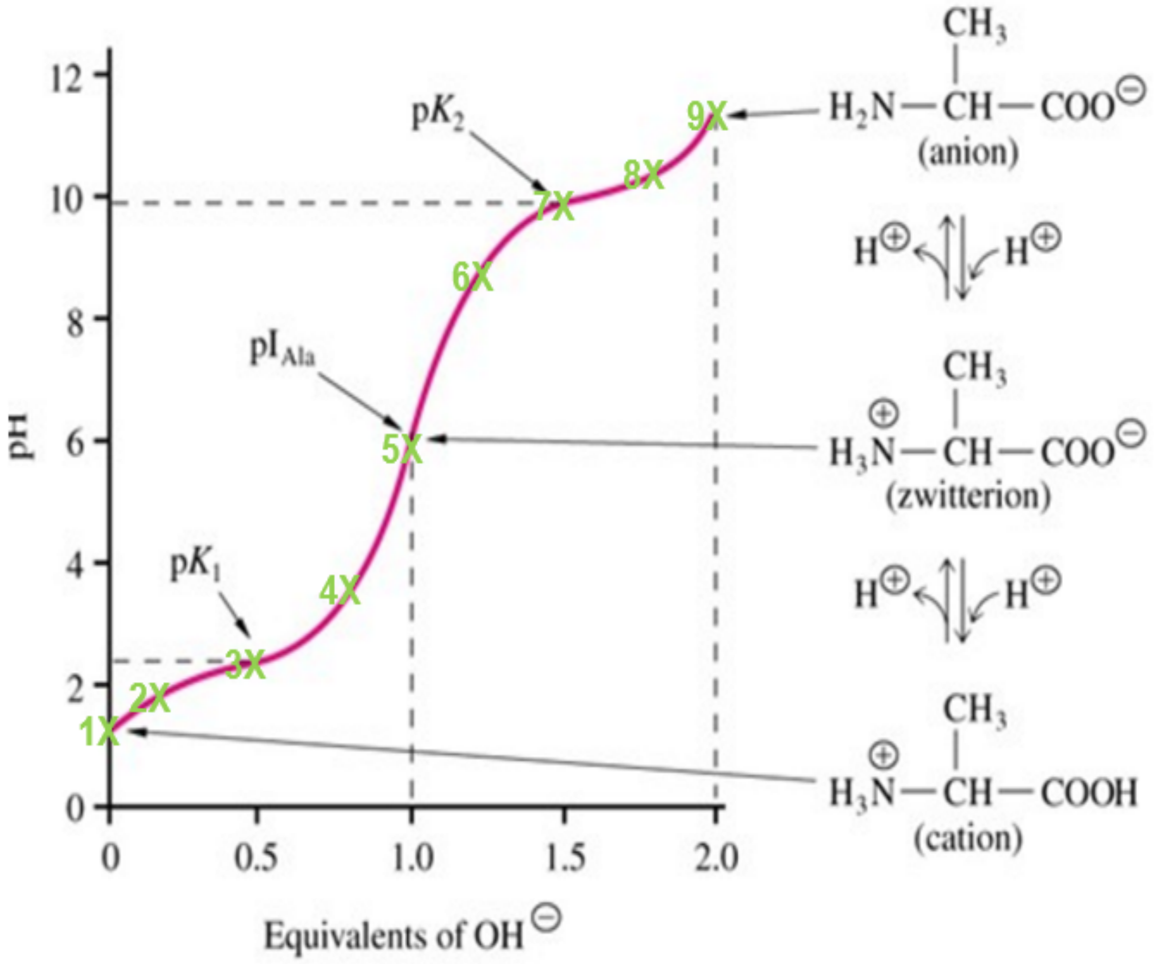
[8] In this point:

- $[NH_3^+\text{-CH-CH}_3\text{-COO}^-] < [NH_2\text{-CH-CH}_3\text{-COO}^-]$.
- $pH > pKa_2$

[9] End point:

- The alanine is full dissociated.
- $[NH_2\text{-CH-CH}_3\text{-COO}^-]$
- pOH= (pkb+P[A-])/2

➔pKb = pKw – pKa2



Calculating the pH at different point of the titration curve :

The pH calculated by different way :

[1] at starting point :

$$pH = (pka + P[HA])/2$$

[2] At any point within the curve (before or in or after middle titration):

$$pH = pka + \log([A-]/[HA])$$

[3] At end point:

$$pOH = (pKb + P[A-])/2$$

$$pH = pKw - pOH$$

$$pKb = pKw - pKa2$$

Practical Part

Objectives:

- To study titration curves of amino acid.
- To use this curve to estimate the pKa values of the ionizable groups of the amino acid.
- To determine pI.
- To determine the buffering region.
- To understand the acid base behaviour of an amino acid.

Method:

a) You are provided with 10 ml of a 0.1M alanine solution, titrate it with 0.1M NaOH adding the base drop wise mixing, and recording the pH after each 0.5 ml NaOH added until you reach a pH=11.

Measured pH value	Amount of 0.1M NaOH added [ml]

b) Take another 10 ml of a 0.1M alanine solution, titrate it with 0.1 M HCL adding the acid drop wise mixing, and recording the pH after each 0.5 ml HCL added until you reach a pH=2.17.

Measured pH value	Amount of 0.1M HCl added [ml]

Results:

- Record the titration table and plot a curve of pH versus ml of titrant added.
- Calculate the pH of the alanine solution after the addition of 0 ml, 5ml, of 0.1M NaOH, and calculate pH after addition of 0.5 ml , 2ml of HCl.
- Determine the pKa of ionizable groups of amino acids.
- Compare your calculated pH values with those obtained from Curve.
- Determine the PI value from your result .